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CURRENT TRENDS IN QUARANTINE ENTOMOLOGY*

Peter A. Follett[1] and Lisa G. Neven[2]
[1] *U.S. Pacific Basin Agricultural Research Center, USDA, Agricultural Research Service, Hilo, Hawaii 96720; email: pfollett@pbarc.ars.usda.gov*
[2] *Yakima Agricultural Research Laboratory, USDA, Agricultural Research Service, Wapato, Washington 98951; email: neven@yarl.ars.usda.gov*

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■ **Abstract** With world trade in agricultural commodities increasing, the introduction of exotic insects into new areas where they become pests will increase. The development and application of quarantine treatments or other mitigation approaches to prevent pest introduction in traded commodities raise many research and regulatory issues. The probit 9 standard for quarantine treatment efficacy has given way to risk-based alternatives. Varietal testing may have merit for some treatments or commodities but not for others. Development of generic treatments to control broad groups of insects or insects in all commodities can expedite new trade in agricultural products. Area-wide pest management programs lower pest levels before harvest and improve the quarantine security provided by any postharvest treatments. Systems approaches capitalize on cumulative pest mortality from multiple control components to achieve quarantine security in an exported commodity. Certain quarantine treatment technologies such as irradiation are not universally accepted, which is slowing their adoption. Standardized phytosanitary measures and research protocols are needed to improve the flow of information when countries propose to trade in a regulated commodity.

INTRODUCTION

World trade in agricultural commodities is growing at a rapid rate. As agricultural trade is increasing, the risk of introducing exotic insects into new areas where they may become plant pests increases. The establishment of new pests can be costly owing to increased crop damage, control programs, and quarantine restrictions on trade. Annual damages caused by exotic insects and mites in the United States have been estimated to be >$17 billion (108).

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A quarantine pest is a plant pest of potential economic importance to an area that is not yet present there or that is present but not widely distributed and officially controlled. Unless accepted disinfestation measures are available, quarantine pests can disrupt marketing of fresh agricultural products not only between countries but also between geographical areas within countries (e.g., Florida to California; Hawaii to U.S. mainland; Queensland to Victoria, Australia; Okinawa to Japan mainland). Therefore, effective postharvest quarantine treatments or pre- or postharvest quarantine systems are essential to the unrestricted trade of fresh and durable commodities through domestic and international channels. Quarantine or phytosanitary treatments eliminate, sterilize, or kill regulatory pests in exported commodities to prevent their introduction and establishment to new areas. As exclusion is the goal for quarantine pests, the tolerance for the pest in the commodity is essentially zero. Although a single postharvest treatment applied to a commodity is still the most common method of quarantine pest control, a range of alternative options, such as multiple or combination treatments, nonhost status, pest-free areas (PFAs), systems approaches, specially designed inspection schemes, and eradication programs, can also be used to prevent exotic pest introductions.

In this review we examine the changing landscape of research and regulatory activities that provide quarantine treatments or other mitigation approaches to reduce or eliminate pest load in traded agricultural commodities. More comprehensive information on invasive species damaging to agriculture, the regulatory framework, and different quarantine treatment methods is provided in several recommended books (42, 107, 121).

POSTHARVEST TREATMENTS

The main procedures for eliminating arthropod pests from a commodity are classified broadly as chemical and physical treatments (107, 121). Chemical treatments include fumigants, such as methyl bromide and phosphine, that penetrate the commodity and are toxic to pests and detergent soaps or insecticides that are used to disinfest commodities of surface pests. Physical treatments include the application of temperature extremes (heat and cold), controlled atmospheres, irradiation, and combinations of these. The application of heat, normally in the form of hot air or hot water, is used to increase the temperature of the host commodity above the thermal limits of survival of the pest, whereas the application of cold is used to decrease the temperature of the host commodity below the thermal limits of survival of the pest. Irradiation breaks chemical bonds in DNA and other molecules, thereby sterilizing the pest or preventing it from achieving sexual maturity. Controlled atmosphere quarantine treatments involve raising the level of CO_2 and lowering the level of O_2 in combination with heat or cold to reduce the duration of the lethal treatment and help maintain commodity quality (16, 98, 100). Other physical treatments such as ozone (61, 75), microwave (65) and radiofrequency heating (94, 126, 134), hyberbaric pressure (14), and vacuum (82) are not currently in use but may have application for certain commodities in the future. Fumigation is the most common type of insect disinfestation treatment, but owing to concerns about the potential effects of fumigants to the environment and human health, physical treatments are expected to replace many of their uses in the future. The development of quarantine treatments is often complicated by commodity sensitivity, particularly fresh commodities, to the available treatment technologies (106, 107).

Traditionally, quarantine treatments were developed for a single pest on a single commodity. Treatment development involved finding a balance between killing the pest and minimizing the adverse effects of the treatment process on commodity quality. Most quarantine treatments for fresh commodities have been developed against internally feeding pests such as tephritid fruit flies because commodities infested by this type of pest are difficult to identify by visual inspection. Many quarantine pests, however, are external or surface feeders, and these pests have equal status with internal pests in interrupting export shipments. Surface-feeding pests do not always require a penetrating quarantine treatment per se, as they can be detected via inspection and culled, or in some cases physical treatments such as high-pressure water or brushes may be sufficient to dislodge the pest from its host (133). A postharvest quarantine treatment may be required for a surface pest if infestations go undetected, such as when individuals are hidden inside fruit clusters (8, 145) or protected within various plant parts in the exported commodity (38, 51, 136).

Probit 9 and Alternative Approaches

Postharvest commodity treatments for pests requiring a high degree of quarantine security are commonly called probit 9 treatments. The reference originates from the statistical method (probit analysis) used for deriving the dose-response relationship. A response at the probit 9 level results in 99.9968% efficacy. The response may be mortality, sterility, or prevention of maturity. The U.S. Department of Agriculture (USDA) has used 99.9968% efficacy as the basis for approving many quarantine treatments, particularly for tephritid fruit flies. A probit 9 treatment usually provides adequate quarantine security, and developing the treatment frequently proves to be the quickest and most easily accepted method for overcoming phytosanitary restrictions.

Probit 9 or 99.9968% mortality is sometimes incorrectly interpreted to mean that 3 survivors are allowed in 100,000 treated insects or 32 survivors in 1 million treated insects (9) without regard for the precision associated with this level of survivorship. To achieve probit 9 mortality at the 95% confidence level, a minimum of 93,613 insects must be tested with no survivors. Quantitative methods have been developed to calculate the number of test insects and confidence limits for other levels of precision and treatment efficacy, with and without survivors (19). Although probit 9 testing seems like a comfortable level of safety, given a highly infested commodity or a high enough volume of infested commodity imports, even probit 9 security could be overwhelmed (85, 109). Other countries (e.g., Japan, Australia, and New Zealand) accept quarantine treatment efficacy at 99.99% (at the 95% confidence level), which is obtained by treating 29,956 insects with no survivors (19). Japan requires 30,000 individuals in three or four trials (124), New Zealand requires three replicates of 10,000 test insects, and Australia accepts 30,000 treated insects with no survivors (55). Statistically, the number of test insects in each replicate is unimportant when there are no survivors but may be important when there are survivors (M. Powell, personal communication). Although it is possible to determine the numbers to be tested in which one or more survivors occur, few countries accept a treatment where there have been failures.

Dose-response testing is used to predict the treatment intensity for large-scale testing; data are typically fitted to a probit model, but another model or transformation (e.g. logit, complementary log-log) may be more appropriate if it provides a better fit to the data (87, 113). During dose-response testing, the normal practice is to estimate treatment response after correcting for control mortality, assuming control mortality is within an acceptable range. Abbott's or a similar formula is usually used (11). However, researchers (and regulators) typically ignore control mortality during large-scale testing and test a predetermined number of insects, i.e., 30,000 or 93,613 insects. Adjustments should be made to the number of treated insects to offset control mortality. Uncertainty in control mortality is also typically not considered, and uncertainty can be considerable for small to moderate control test size. The size of the adjustment due to uncertainty depends on both the observed proportion of survival in the control and the control test size. For example, Monte Carlo simulations estimate that the number of treated insects needed during large-scale testing with no survivors increases from 30,000 (99.99% mortality at the 95% confidence level) to 33,500 when survival is reduced from 100% to 90% in a small-scale control test with 100 insects (M. Powell, personal communication). If the size of the control test is 5000 insects with 90% survival, 33,000 insects must be tested. When control survival is decreased to 60% with 5000 insects, the number of treated insects with no survivors must be increased to approximately 50,000 individuals.

In certain cases, lower numbers of insects may be acceptable during quarantine treatment development if the potential economic and environmental impact of the pest should it be introduced are low. For example, irradiation treatment with a dose of 300 Gy was accepted for the mango seed weevil, *Sternochetus mangiferae* (26), a monophagous pest of mangos, on the basis of evidence for the weevil's limited potential impact on U.S. agriculture (32) and cumulative data from several studies with a few thousand insects showing prevention of adult emergence from the fruit at this dose and sterilization at lower doses (29, 55, 120).

As mentioned above, many postharvest treatments negatively affect commodity quality. Therefore, reducing the severity of a quarantine treatment may improve the shelf life or marketability of the commodity. Landolt et al. (79) pointed out that the probit 9 standard may be too stringent for commodities that are rarely infested or are poor hosts, and hence a less severe postharvest treatment might still provide quarantine security. The alternative treatment efficacy approach measures risk as the probability of a mating pair or reproductive individual surviving in a shipment. This will be a function of many factors including infestation rate, culling and other postharvest removal of infested fruit, shipment volume, shipping and storage conditions and the mortality these conditions exact on the pest, and other biological and nonbiological factors (129, 140). The probability of establishment after shipment is a function of many additional factors including host availability and suitability of the climate (137, 139). The main quantitative argument for deviating from probit 9 treatment efficacy is low infestation rate of the commodity. This approach makes an important conceptual advance by focusing on absolute numbers of survivors rather than percent mortality. Maximum pest limit is another concept closely related to the alternative treatment efficacy that focuses on survival rather than mortality (10, 85). It is defined as the maximum number of insects that can be present in a consignment imported during a specified time at a specified location, and is therefore flexible in terms of treatment efficacy and permissible infestation levels (10).

A number of quarantine pest-commodity systems are amenable to the alternative treatment efficacy approach (34, 81). For example, nectarines are an inherently poor host for codling moth, *Cydia pomonella*. Only three live codling moths (larvae) were found infesting 326,625 packed nectarines sampled from packinghouses in the San Joaquin Valley of California for an infestation rate of 9.2×10^{-6} (21). In an average shipment of 16,000 kg (89,600 fruits), the probability of one or more mating pairs surviving after a probit 9–level quarantine treatment is 1.7×10^{-10}. The actual mortality level required to prevent a mating pair of codling moths in a single shipment of nectarines with 95% confidence is 77.74% (probit 5.65). Hypothetically, if 100 shipments arrived at the same location, the probability of one or more codling moth mating pairs surviving in nectarines after a probit 9–level quarantine treatment is still extremely small (1.7×10^{-6}). In this case, a probit 9 treatment provides a high level of overkill, and a less severe treatment might be developed that provides adequate quarantine security while minimizing the adverse effects of the treatment on commodity quality. Low infestation rate at harvest can also be the result of effective pest management before harvest or the harvest of climacteric fruit (those that continue to ripen after harvest) at a nonpreferred maturity stage (discussed below). An advantage to using the alternative treatment efficacy approach is that fewer insects may be needed during research to develop quarantine treatments (34).

Varietal Testing

Evaluation of the efficacy of quarantine treatments to control pests of regulatory concern may include a requirement to determine pest response to the treatment on different varieties of the commodity proposed for export. These tests are often called varietal testing.

For irradiation, varietal testing (i.e., comparisons among varieties with divergent physical properties) may be important during development of a quarantine treatment. O_2 concentration modifies sensitivity to irradiation, and conditions producing hypoxia can increase radiation tolerance (1). Fruit flies have higher radiotolerance when treated in a nitrogen atmosphere than when treated in ambient air (28), and when treated in fruit compared with artificial diet (31). Radiation damage and mortality were less in codling moth larvae treated in 0.25% O_2 compared with 3% O_2 (12). Theoretically, varieties of a commodity with higher water content may have lower available O_2, and insects infesting these varieties would show higher radiotolerance. Variety had a dramatic effect on egg hatch and larval development during irradiation studies with Mediterranean fruit fly, *Ceratitis capitata*, in nectarines (eight varieties) and plums (four varieties) (74), and a link with fruit moisture content was suspected but not measured.

Varietal testing is essential during development of certain quarantine treatments or approaches. Heating and cooling properties may differ significantly among varieties that vary greatly in size, shape, or density. Approved hot water immersion treatments for mangos to kill fruit flies vary depending on fruit size and whether the variety is rounded or flat (128), and treatment of oversize mangos may result in survivors (122). Also, varieties often differ in their susceptibility to insect infestation, e.g., avocado susceptibility to Caribbean fruit fly, *Anastrepha suspensa* (60), which is important during nonhost testing.

For fumigants, Bond (13) suggested that if c × t (concentration × time) values differ depending on the variety, the efficacy of a treatment might vary according to varieties. Varietal testing was conducted for codling moth response to methyl bromide fumigation in nectarines. Yokoyama et al. (143, 144) reported differences among nine nectarine cultivars in LC_{50} values for 1-day-old codling moth eggs treated with methyl bromide when nonoverlap of 95% confidence limits was used to determine significance, but reanalysis using response ratios found no significant differences and suggested differences in insect response among cultivars reflected natural variation (115). Variety was not an important factor in studies on codling moth response to methyl bromide in walnuts (52, 53), apples (48), and sweet cherries (47, 90). Codling moth researchers in New Zealand found differences in sorption of methyl bromide between cherry varieties, but they also found strong differences between seasons for the same varieties, suggesting the condition of the fruit or another factor might be more important than cultivar per se (84). Methods of measuring fumigant concentration may contribute to variable results. Fumigant concentration is typically measured by drawing gas samples from the air surrounding the commodity, which may lead to inaccuracies in measuring the level of gas experienced by insects feeding inside the commodity. Also, some researchers calculate c × t values at a single time during the fumigation, while others use an integration of c × t values over time. Nevertheless, there may be basic differences in how fruit firmness, water content, and other physical parameters affect sorption of methyl bromide and other fumigants. Varietal tests in general should estimate insect response using multiple randomly selected cohorts from a population or intergenerational studies, so that natural variation can be separated from other factors (114).

Generic Treatments

The generic quarantine treatment concept has several definitions. It can mean a treatment that provides quarantine security for a broad group of pests without affecting the quality of a wide range of commodities (133), a treatment for all pests infesting a single commodity (128), or a treatment for a single pest on many different commodities (30). Here we focus on the first definition.

There is evidence for and against the generic treatment concept depending on the treatment. For most treatments, an ultra-severe regimen could be devised that is universally lethal to insects. In many cases, this treatment would also likely be detrimental to commodity quality. Treatment development involves finding the balance between eliminating live pests and minimizing the adverse effects of the treatment process on commodity quality. New Zealand Ministry of Agriculture and Forestry (MAF) has promoted the acceptance of a generic heat treatment against tephritid fruit flies (93). Disregarding the impact on the commodity, the contention is that, for a given pest, a single heat treatment should be acceptable for all commodities it infests. Insect thermal tolerance modeling is based on this premise (68, 71). This approach neglects several important concepts in thermal quarantine treatments. Heat and cold treatments must consider (*a*) commodity tolerance, (*b*) insect tolerance, (*c*) preharvest thermal experience of the insect and commodity, and (*d*) the rate of heating or cooling (96). Although modeling may provide useful information on relative thermal tolerances, measuring insect thermal responses in the heated commodity is essential. For example, Mediterranean fruit fly can infest hundreds of commodities, from grapes to grapefruits; for a given treatment protocol, Mediterranean fruit fly may respond differently in different fruit because of the heating rate. The milieu surrounding the insect can also influence mortality during treatment development. The estimated LT_{95} was 21% to 30% lower for third-instar Caribbean fruit fly exposed to heat (43°C) in grapefruit juice compared with exposure to the same temperature in tap water or water adjusted to a pH equal to grapefruit juice (40). Mangan et al. (87) suggested that a minimum fruit center temperature of 44°C sustained for 100 min was sufficient to provide probit 9 mortality at 95% confidence levels of *Anastrepha ludens* in grapefruit, tangerine, and navel and Valencia oranges. Their observations were made after the treatments had been developed using maximum ramping rates.

Generic thermal treatments are complicated by acclimatization and acclimation in both the insect and commodity (95, 96). Acclimatization is the modification of an organism's physiology in response to natural environmental change. In the field, thermal fluctuations are common and both insect and host commodity modify their physiology in response to these fluctuations. Acclimation refers to the ability of an organism to respond to a short-term or acute thermal change. Acclimatization or preconditioning may affect acclimation. Most quarantine treatments are developed with laboratory-reared insects that rarely experience thermal fluctuations, and then the treatment is applied to wild insects that do experience natural thermal fluctuations. In this case, insect acclimatization could result in thermal treatment failure (86).

Modification of thermal quarantine treatments can override the effects of acclimatization. Controlled-atmosphere temperature treatments prevent thermal acclimation by the addition of a low-O_2, elevated-CO_2 environment along with the heat treatment (97). Under controlled-atmosphere conditions, the production of heat shock proteins by insects is inhibited, indicating a compromise of the acclimation process (L. Neven, unpublished data). A thermal heat treatment in which the rate of temperature change exceeds the insect's ability to acclimate (127) may also be amenable to application as a generic heat treatment.

The thermal history of the commodity or the insect pest could compromise postharvest treatments developed under static temperature conditions. Waddell et al. (132) calculated the extra time it would take to kill eggs of the fruit fly *Bactrocera tryoni*, if it experienced high, sublethal temperatures prior to a lethal heat treatment, and examined the effects of various heating rates on treatment efficacy. The thermal conditions experienced by the insect prior to treatment, particularly in the 32 to 42°C range in which insect thermal conditioning occurs, greatly influenced mortality. For example, the estimated LT_{99} for *B. tryoni* eggs immersed in water at 46°C increased from 18.9 min with no preconditioning to 45.1 min after a 1-h conditioning treatment at 38°C. Similarly, insect-rearing temperature is positively correlated with survival during heat treatments (39).

As with insects, commodity tolerances can be influenced by the rate of heating, final treatment temperature, and duration of the treatment (99, 101), and thermal experience by the commodity prior to harvest can also affect tolerance (138).

Currently accepted heat treatments for commodities coming into the United States (Table 1) often do not specify the rate of increase to the target temperature, or ramp time, which may cause some problems due to insect acclimation for treatments with a slow heating rate. For a number of treatments, a hold time at the final target core temperature is also not specified. These treatments may have been developed to provide overkill by exceeding the insect's ability to handle the maximum heat load, but with the development of more thermal treatments, treatment specifications need to be more exacting and also consider both field experience and commodity size, which would mean specifying a target temperature hold time and a specified heating rate.

Irradiation does not affect the quality of most commodities at dose levels that are effective against most insects and mites and therefore may be ideal for developing generic quarantine treatments. Before generic irradiation treatments can be recommended, information is needed on effective doses for a wide range of insects within a taxon, guild, or pest group. For example, a generic treatment could be developed for tephritid fruit flies in the genus *Bactrocera*, for weevils in the family Curculionidae, or for stored-product insects. In 1986, the International Consultative Group on Food Irradiation proposed generic irradiation doses of 150 Gy for tephritid fruit flies and 300 Gy for all other insects (66). The concept of generic irradiation doses has been used since 1997 to a limited extent for a variety of

TABLE 1 Currently approved heat treatments for the control of fruit flies in commodities exported to the United States

Treatment	Target	Center temp.	Ramp time	Holding time	Origin	Commodity
Hot water	*Anastrepha* sp., *C. capitata,* *B. dorsalis,* *B. cucurbitae*	NS	NS	NS	Mexico, Hawaii	Mango, lychee
HFA	*Anastrepha* sp.	118°F (48°C)	210 min	NS	Mexico	Grapefruit
HFA	*A. ludens,* *A. obliqua,* *A. serpentina*	118°F (48°C)	NS	NS	Mexico	Mango
HFA	*C. capitata,* *B. dorsalis,* *B. cucurbitae*	117°F (47.2°C)	240 min	NS	Belize, Hawaii, Chile	Papaya, mountain papaya
Vapor	*Anastrepha* sp.	110°F (43.3°C)	480 min, *360 min	360 min, *240 min	Mexico	*Clementine, grapefruit, mango, *orange
Vapor	*C. capitata,* *B. dorsalis,* *B. cucurbitae*	118°F (44.4°C)	PPQ officer	525 min	NS	Bell pepper, eggplant, mountain papaya, pineapple, squash, tomato, zucchini
Vapor	*B. dorsalis*	115.7°F (46.5°C)	NS	30 min	Taiwan	Mango

Source: Reference 128.

HFA, hot forced air.

NS, not specified.

tropical fruit pests in Hawaii, despite information gaps (31). The United States is recommending a 150 Gy irradiation dose for tephritids on the basis of information from 17 quarantine species in four genera (*Anastrepha*, *Bactrocera*, *Ceratitis*, and *Rhagoletis*) (33, 41, 54). New Zealand is proposing generic doses of 150 Gy for tephritid fruit flies, 250 Gy for other insects, and 300 Gy for mites of tropical fruits exported from Australia (18).

The high dose approach is a variation on the generic dose concept. With this approach a dose is set in excess of that believed to be required to control the pests associated with the commodity. For example, a high irradiation dose of 400 Gy was approved for two sweet potato pests in sweet potatoes exported from Hawaii to the U.S. mainland (27). The two regulatory pests, West Indian sweetpotato weevil, *Euscepes postfasciatus*, and sweetpotato vine borer, *Omphisa anastomosalis*, are in the families Curculionidae and Pyralidae, respectively, and data from the irradiation literature on other curculionid and pyralid pests suggested this dose would be adequate (63).

Theoretically, a generic or universal irradiation dose could be set for all insects. The most radiotolerant insect species reported to date is the Angoumois grain moth, *Sitotroga cerealella*, a stored-product pest that successfully reproduced at

500 Gy but not at 600 Gy (64). If this is indeed the most tolerant insect, irradiation treatment with a minimum absorbed dose of 600 Gy should control any insect. A limiting factor for the practical use of a generic treatment at 600 Gy in the United States is the 1000 Gy (1 kGy) maximum allowed dose for fresh produce set by the Food and Drug Administration. With typical dose uniformity ratios of 1.5 to 3.0 at commercial irradiation facilities, treatment to achieve a minimum absorbed dose of 600 Gy without exceeding 1 kGy would be difficult. Also, doses above 600 Gy adversely affect the quality of many fresh commodities (72, 91). Another approach would be to set the generic dose for insects at a dose lower than 600 Gy and exclude any species or insect groups that tolerate a dose above this level (31). For example, a generic irradiation dose of 400 Gy for arthropods is supported by available data if Lepidoptera pupae and adults, and mites, are excluded (33); the 400-Gy generic dose would reduce the problem of exceeding the 1 kGy limit.

Generic methyl bromide treatments have been developed for a broad range of insects and commodities (128). For example, 2 lb methyl bromide per 1000 ft^3 of container volume for 2 h at 70°C or above is an approved treatment in the United States for various groups of external and internal feeders including leafminers, mites, noctuids, pentatomids, tephritids, thrips, and tortricids on more than 40 fruits and vegetables. Certain commodities and pests require higher concentrations of methyl bromide. This fumigant is broadly effective against insects and mites, has excellent penetration characteristics, and is tolerated by many commodities.

ALTERNATIVES TO POSTHARVEST TREATMENTS

Although a single postharvest treatment applied to the commodity is still the most common method of quarantine pest control, a range of alternative analytical techniques and mitigation options exists to prevent exotic pest introductions. Alternative approaches such as multiple or combination treatments, nonhost status, PFAs, pest eradication, systems approaches, and a variety of specially designed inspection schemes can also provide the basis for establishing quarantine security. Below we discuss several approaches to quarantine pest exclusion and provide examples to illustrate each approach.

Nonhost Status

A commodity may be exported if it is proven to be a nonhost for all or part of its growth cycle. Armstrong (5, 7) defined a fruit fly host as a fruit or vegetable onto which an insect deposits eggs, the eggs hatch into larvae, and the larvae feed and develop to form viable pupae from which adults emerge. If the insect cannot completely develop to form viable adults the plant is a nonhost. Hawaiian “Cavendish” bananas are approved for export to the U.S. mainland as nonhosts for Mediterranean fruit fly, *Ceratitis capitata*, and oriental fruit fly, *Bactrocera dorsalis*, when harvested in the mature green stage and free of blemishes, although ripe fruit are preferred hosts (8). Lychee and longan are shipped from Florida to California as nonhosts for Caribbean fruit fly, *Anastrepha suspensa* (37). A broad definition for a nonhost that applies more generally to insects would be a host on which the insect cannot develop to sexual maturity and successfully reproduce.

The physiological basis for host nonpreference or nonsuitability by a quarantine pest is often not understood; therefore, establishing nonhost status can be difficult, as researchers must conduct infestability studies under a wide range of conditions and over multiple years. Also, sufficient numbers of fruit or insects must be included in the study to separate true nonhosts from poor hosts or rarely infested hosts (see below). The importance of year-to-year variation and research methodology was illustrated in the development of a nonhost protocol for Hawaiian "Sharwil" avocados. In the laboratory, Sharwil avocados with stems attached were not susceptible to fruit fly infestation for up to 12 h after harvest but then became good hosts (6). Inspection of >114,000 harvest mature fruit over two seasons indicated no fruit fly infestations, and the data were used to approve a nonhost status export protocol from Hawaii. A later study (105) showed that Sharwil avocados became infested albeit at low levels when Mediterranean fruit flies and oriental fruit flies were caged with fruit still attached to the tree, casting doubt on the nonhost status. In 1992, live larvae were found in fruit samples from orchards and the protocol was suspended. In the first year of a follow-up study, oriental fruit flies were found in 15 of 3248 harvest-ripe Sharwil avocados collected off the tree, but in the second year 0 of 5004 fruits were infested (80). Of the 15 infested fruit, 5 fruit had no indications of infestation, emphasizing the difficulty in predicting the occurrence of fruit fly infestation in mature green avocados. This study also showed that "firm ripe" and "fully ripe" fruit occur on the tree (2.2%) late in the season and are much more likely to be infested than mature green fruit. The mechanism of resistance in avocado against fruit flies is unknown.

Pest-Free Areas

PFAs are officially identified or established areas in which a target pest does not occur and is maintained as such (24). PFA status is aimed at designated commodities from specific geographic areas on the basis of the absence of a specific pest or pest complex. The basis for accepting a PFA is a sound pest risk assessment combined with strong evidence of effective surveillance and exclusion measures to maintain the areas pest free. Sensitive survey tools for detection of the target pests must be available. The earliest and longest standing PFA program was established in Chile in 1982, and more than 25 fruits are approved for export from the PFA to the United States, including apple, apricot, avocado, cherry, kiwifruit, nectarine, peach, pear, and persimmon. The Chilean program centers on an effective surveillance program for exotic fruit flies (Mediterranean fruit fly and *Anastrepha* spp.), a strong exclusion program, and immediate implementation and successful completion of emergency procedures each time adult Mediterranean fruit flies or other fruit flies are detected.

To establish and maintain PFAs, the rule of thumb is that detection devices should detect incipient populations before they reach the third generation and spread beyond a radius of 2.5 km so that new infestations can be delimited and controlled to maintain the PFA (81). This typically involves establishing a trap array, and beforehand it must be proven that trap data accurately reflect the pest situation in the field. In the case of fruit flies, trapping systems are validated by comparing the number of adults caught in traps with the number of larvae detected by cutting fruit open. Based on information from trap arrays, regulatory officials decide whether to continue certification, suspend certification, or initiate pest suppression measures to bring the area back into compliance (112). Suppression is usually achieved with insecticide or insecticide-laced bait sprays, but sterile-insect release has also been used. Efforts to establish or demonstrate an area is pest free are greatly enhanced when geographic barriers such as ocean or mountains help exclude the pest, when the area is isolated from urban areas, and when the commodities to be exported are poor or rarely infested hosts of the target pest. A pest-free period may be used as a temporal barrier to infestation; early-season stone fruits are exported from California before the quarantine pest *Rhagoletis completa* emerges and begins ovipositing (141, 142). Maintaining the identity of the fruit harvested from certified areas to prevent mixing with fruit from noncertified areas is another important element to the program. One of the main considerations whether to establish and maintain a PFA is economics. High costs are associated with development of the program and with ongoing surveillance and regulatory measures.

Systems Approach

The systems approach integrates many biological and physical factors with operational procedures to cumulatively provide quarantine security (69, 70). A postharvest treatment may be one component of a systems approach. In general, systems approaches are more difficult to manage than a postharvest treatment alone or a PFA because many of the components need to be supervised or monitored to ensure compliance (81). The components of the systems approach can vary widely but commonly include pest survey, trapping and sampling, field treatment, cultural practices, host resistance, postharvest safeguards, limited harvest period, limited sales distribution, and restrictions on crop maturity at harvest. For example, the USDA prohibits import of bell peppers from areas where Mediterranean fruit fly occurs; bell peppers are permitted from Israel with a systems approach that includes growing the host within a fly-proof greenhouse, greenhouses located in areas where Mediterranean fruit fly is absent or rare, trapping the surrounding area, and fly-proof packaging. Citrus fruits are shipped from Florida to other states and foreign locations using a systems approach to prevent infestation by

Caribbean fruit fly that includes poor host status, removal of alternative hosts, established growing areas with buffers, trapping, field treatment, restricted harvest periods, and fruit cutting (112). Papayas are imported into the United States from Brazil and Costa Rica with special conditions for growing, treating, packing and shipping, field sanitation, fruit fly trapping, and issuance of a phytosanitary certificate (25). The European and Mediterranean Plant Protection Organization recommends various systems approaches for many of its quarantine insect pests involving different combinations of phytosanitary certification, inspection, area freedom, and, in some cases, postharvest treatment using fumigation, heat, or cold (15).

Multiple safeguards provide redundancy so that if one mitigating measure fails other safeguards exist that still reduce the risk to a negligible level. Because systems approaches rely heavily on a sound knowledge of the pest and host biology and how they relate to each other, the programs can be time-consuming and costly to develop. Also, the systems approach is vulnerable to breakdown when the economics of pest control or monitoring are altered. For example, a systems approach against oriental fruit moth, *Grapholita molesta*, in peaches and nectarines grown in California and exported for Mexico operated for a number of years until 2004, when the Mexican government added another 22 pests to the quarantine list, making adherence to a systems approach unattainable (R. Neenan, personal communication). Despite the limitations, a systems approach may be practical when the host-pest relationships are well understood and can be manipulated; when multiple mitigation measures that reduce pest infestation are available; when available postharvest treatments alone are impractical owing to time requirements or expense, or when they cause detrimental effects to the commodity; or when targeting organic produce markets. In terms of host-pest relationships, systems approaches are particularly attractive for commodities that are poor hosts for the quarantine pest and when the distribution of the quarantine pest is limited or the pest can easily be excluded from the area where the commodity is grown.

A risk management option that has not been exploited is shipment volume (34, 129). In fewer or smaller shipments there are fewer insects, and the probability of having infested commodity and surviving insects is lower compared with more frequent and larger shipments. A maximum allowable shipment volume for a commodity arriving at a location over a predetermined period could be part of an export regulation in the same manner in which limited distribution period and limited geographic distribution are currently used.

A systems approach fits well with the alternative treatment efficacy approach discussed above. For example, irradiation of avocados at doses providing probit 9 level of kill of tephritid fruit flies and other pests ($\geq$150 Gy) causes discoloration to the fruit flesh. In Hawaii, oriental fruit fly is the main quarantine pest of avocados. Whereas 120 Gy is required to give probit 9 kill of oriental fruit fly, irradiation treatment at a dose of 80 Gy provides >99% kill (31) and could be combined with cold storage, poor host status, inspection, field control, or other mitigation procedures to result in a high level of quarantine security.

Pest Eradication

Eradication is the elimination of all individuals of a species from a geographic area where reinvasion is unlikely to occur (92). Researchers have attempted pest eradication using a variety of tactics including sterile-insect technique (SIT), male annihilation, insecticides or insecticide-laced bait sprays, biological control, or combinations of these tactics. The eradication of a pest from an agricultural region is technically challenging and, depending on the methods used, can be a socially charged proposal. Eradication in urban areas using aerial and ground application of pesticides can generate public opposition, whereas SIT, mass trapping, or male annihilation programs in the same areas engender little or no public concern (23). Eradication, regardless of which method is used, is ultimately determined by the pest and the environment. In situations in which the insect is invasive and not established in the environment, immediate and aggressive eradication efforts may result in success. However, when the pest becomes widely dispersed and established, eradication is much more problematic (92). In this latter situation, area-wide suppression is more practical. Successful programs are the eradication of the screwworm, *Cochliomyia hominivorax*, from the United States and Mexico (110); the cattle tick, *Boophilus annulatus,* which carries cattle tick fever, from the United States (76); the tsetse fly, *Glossina austeni*, from Zanzibar (131); the oriental fruit fly, *B. dorsalis*, from Okinawa and the Mariana Islands (78); white spotted tussock moth, *Orgyia thyellina*, from New Zealand's North Island (62); the Queensland fruit fly, *B. tryoni*, from Western Australia; and the melon fly, *B. cucurbitae*, from the Kyukyu Archipelago of Japan (58). Various eradication techniques are described below.

STERILE-INSECT TECHNIQUE SIT has been the most widely used eradication strategy (57). Sterile males are released in large numbers into the field where they mate with feral females, thus interfering with reproduction and leading to population decline. The assumptions underlying a successful SIT eradication program are that the insects can be reared and sterilized in large numbers; sterile insects can be distributed so that they mix thoroughly with the wild population; sterile insects compete successfully for mates; the release ratio is sufficiently large to overcome the natural rate of population increase, so that the trend in population size is downward after the first release; and the target population is closed—there is no immigration of fertile insects from outside the release area. SIT may be ineffective if population regulation exhibits nonlinear density dependence and if population numbers rebound after a release. Also, if the population consists of many subpopulations and some populations are not accessible to released sterile insects, migration among subpopulations may prevent eradication (36).

Many innovations are being made in SIT technology to improve production efficiency (88, 117, 116) and male competitiveness (123) and thereby increase induced sterility in the field (111). SIT is used throughout the world on a variety of pests such as screwworm, Mediterranean fruit fly, several species of *Bactrocera* and *Anastrepha* fruit flies, tsetse fly (*Glossina* spp.), *Anopheles* mosquito, pink bollworm (*Pectinophora gossypiella*), and codling moth (*Cydia pomonella*) (57, 58, 76).

Cost-benefit analyses support the continued expansion of SIT for eradication or suppression of key pests (58). The investment of nearly \$1 billion between 1957 and 2000 on screwworm has resulted in annual direct producer benefits estimated at >\$1 billion. Eradication of Mediterranean fruit fly from Mexico and maintaining the country free of the pest at a cost of \$8 million per year protects a \$1 billion per year fruit and vegetable export industry and prevents movement of the fly into the United States. The eradication of Mediterranean fruit fly from Chile is predicted to open markets for fruit exports worth \$500 million per year (57).

MALE ANNIHILATION With male annihilation, fruit flies are killed by insecticide-laced lures that release an attractant to draw in male flies. Eradication programs targeting oriental fruit fly in Okinawa and the Mariana Islands are primarily based on male annihilation and involve distribution of coconut husks soaked in methyl eugenol (attractant) and malathion (insecticide) at a density of 400 blocks per km^2. Eradication efforts for melon fly and mango fruit fly in these islands use the lure cuelure and the insecticide fipronil applied to fiberboard blocks at a rate of 1000 blocks per km^2 (78, 125). Eradication using male annihilation or SIT may be difficult for pests distributed over large areas with high fecundity, high vagility, and many noncrop hosts. These tactics are still useful for area-wide pest suppression and could be used as part of a systems approach for control of a quarantine pest (130).

AUTOCIDAL BIOLOGICAL CONTROL Autocidal biological control (ABC) is currently a theory but could soon become a reality. The term, originally coined by Fryxell & Miller (35), was based on an idea from Knipling's group (77), in which they proposed to develop a nondiapausing strain of *Diabrotica* and perform overflooding releases in a type of self-killing (autocidal) pest control program. Genetically engineering insects using transposable elements such as *Hobo*, *piggyBac* (17, 43, 44, 56), *Hermes* (89, 104), and *minos* (83) facilitates strain development. With these high-efficiency transposable elements available for insect transformation, it has been proposed that pest insects can be transformed with conditionally lethal genes that would be controlled under laboratory conditions to allow for mass rearing and become lethal under normal environmental conditions (35). ABC operates in the same manner as SIT but does not require the application of a sterilizing dose of irradiation.

Simulation models have been used to explore ways to improve conditional lethality using genetically modified insects carrying gene insertions at multiple loci, gene insertions causing female specific lethality, or gene insertions causing sex ratio distortion (36, 118, 119). In all cases, the genetically modified insects potentially were orders of magnitude more efficient at reducing population levels than were traditional sterile male releases. For example, a single release of 19 males carrying the female-killing gene for every two wild males, with the female killing trait on 10 loci, would reduce the population to 0.002% of a no-release population, whereas a sterile male release of equal size would reduce the population to 5% of a no-release population. These models are theoretical but point to the potential strengths of a transgenic approach to insect population control.

PHYTOSANITARY RULES AND TRADE

The 1994 North American Free-Trade Agreement (3), and the Agreement on Sanitary and Phytosanitary Measures of the 1995 General Agreement on Trades and Tariffs, Uruguay Round (4), contain elaborate international rules that govern the use of phytosanitary regulations in trade. Generally, these agreements require that governments adopt phytosanitary measures, including import restrictions, treatments, and other border control measures that affect trade in an open, nondiscriminatory, and scientific fashion (73).

New concepts such as risk assessment, regionalization, equivalence, and transparency are now part of the vocabulary of international agricultural trade. Risk assessment is the evaluation of the likelihood of entry, establishment, or spread of a pest or disease within the territory of the importing nation according to the phytosanitary measures that might be applied, and of the associated potential for biological and economic consequences (22). The idea of regionalization is a concept used to recognize that certain areas or regions present a low pest or disease risk and therefore trade in fresh produce from those areas should proceed unimpeded. Plant quarantine officials often use the synonymous terms "area freedom" or "pest-free area." Equivalence means that countries are required to recognize another country's phytosanitary measures, though they may be different, as equivalent to their own when the exporting country demonstrates that its treatments or pest control procedures provide the importing country's desired level of quarantine security. This is not a new idea but it is often not applied consistently. For example, the application of irradiation for phytosanitary purposes is prohibited in Japan and the European Union. Transparency refers to the requirement that countries provide information about new or changing phytosanitary measures that may affect trade and provide countries an opportunity to comment on proposed rules (73).

Universal Acceptance of Quarantine Technologies

Not all quarantine treatments or approaches are universally accepted. For example, Australia has actively pursued PFA technologies to satisfy quarantine restrictions on pest fruit flies. For many years, some countries did not recognize the limited distribution of the Mediterranean fruit fly in Australia (PFA concept) but declared the entire country as part of the insect's distribution. This either restricted market availability or led to the development of treatments against this pest for application to products that come from uninfested areas. The lack of recognition of PFAs distorted Australia's quarantine status. Likewise, codling moth does not occur in Western Australia, and official internal quarantines are in place to maintain this status. Nevertheless, some countries designated all of Australia as a codling moth–infested area and did not accept apples and pears on this basis (M. Stuart, personal communication). Certain quarantine technologies such as irradiation are controversial. Japan irradiates potatoes for sprouting control but does not permit the import of irradiated produce or other food products. The European Commission allows irradiation of spices, herbs, and vegetable seasonings by members of the European Union but prohibits the use of irradiation as a phytosanitary measure (33).

A number of commodities are so rarely infested by a quarantine pest that they probably do not require a quarantine treatment. For example, sweet cherry is a very poor host for codling moth (45, 46, 49, 50), and some believe it is a nonhost (135). In sweet cherries from California and the U.S. Pacific Northwest in 1997, four suspected codling moth larvae were found in more than 218 million inspected cherries exported to Japan, an infestation rate of 1.8×10^{-8}, and in 1998 and 1999, no codling moth larvae were found in more than 423 million inspected cherries (34). Despite this low probability of infestation, Japan requires methyl bromide treatment of U.S. sweet cherries before export.

One problem with accepting another country's data for a quarantine treatment or approach is that the methods for generating the information are poorly defined or have not been rigorously debated. For example, Hass avocados in Michoacan, Mexico, were recently reported as a nonhost for *Anastrepha* fruit flies (2). Methods to establish nonhost status in this study followed those recommended by New Zealand MAF (20), the only published nonhost standard at this time. The New Zealand MAF host-testing protocol begins with a laboratory cage trial that involves exposing 500 g of fruit to a number of gravid females to ensure that 250 to 500 eggs are laid, replicated five times. In assessing the results, if adults are reared from a single control replicate of a known host fruit exposed to gravid females and no adults are reared from the five replicates of trial fruit, then the trial fruit is declared a nonhost and further testing is unnecessary. This means nonhost status could be determined by testing as few as 1250 eggs and <100 fruit. If fruit nonpreference or antibiosis are considered equivalent to a quarantine treatment, this level of testing is far below the traditional statistical standards (99.99% to 99.9968% mortality at the 95% confidence level). The level of confidence associated with treating a number of insects with zero survivors is given by the equation $C = 1 - (1 - p_u)^n$, where p_u is the acceptable level of survivorship and n is the number of test insects (19). If we assume that 99.99% mortality is required ($p_u = 0.0001$), the level of confidence associated with testing 2500 insects with 0 survivors is 22.1%. Other studies leading to nonhost protocols have used considerably more insects and fruit. Nonhost status of "Tahiti" limes for Caribbean fruit fly was demonstrated after inspecting 102,384 unsorted, ungraded packinghouse fruit from 184 different groves on 60 harvest dates and finding no infested fruit (59). Sharwil avocados were declared nonhosts for oriental and Mediterranean fruit flies after inspecting more than 114,112 fruits during two seasons with no observed infestation (6). Nonhost status of lychee and longan for Caribbean fruit fly was demonstrated by exposing 34,016 fruit under laboratory or field conditions with no adult emergence (37).

Standardized Phytosanitary Measures and Research Protocols

Phytosanitary regulations have been used as a barrier to trade and often lack a sound scientific foundation. This problem is being addressed by the International Plant Protection Convention (IPPC) through the establishment of international standards (67). By participating in standard-setting processes and observing internationally accepted standards, governments are better able to achieve an appropriate level of protection while reducing the likelihood of trade challenges. The emphasis in standards on feasibility and technical soundness ensures that the same principles and procedures are beneficial where trade is not the primary concern. Recent IPPC standards have been published, giving guidelines or requirements for pest risk analysis, PFAs, pest eradication, and integrated measures for a systems approach to pest risk management (66).

Future trade between countries in a commodity that is potentially infested by a quarantine pest can be slowed by the lack of a standardized research protocol for developing a quarantine treatment or system. The exporting country must often initiate research on a crop or quarantine pest without full knowledge of the commitment of time and resources involved because the importing country has not published or explicitly outlined a research protocol. Research requirements can vary dramatically depending on the pest, the crop, and the country.

Research for developing quarantine treatments frequently has had some shortcomings, including inadequate sample size, failure to treat the most tolerant stage, treatment of larvae in air or in diet rather than in the commodity, inadequate or unreported treatment parameters, an insufficient number or range of treatment doses, incomplete or inexact reporting of the experimental methods, and the absence of large-scale tests (31). These shortcomings make evaluation and comparison of the results and conclusions from different studies difficult. Standardized research protocols help ensure that quarantine research is of uniformly high quality and that results from different commodities and for different pest species are comparable. Such standards must be comprehensive yet flexible to consider all potential quarantine insects, commodities, and conditions.

The IPPC recently published guidelines for the use of irradiation as a phytosanitary treatment that included an appendix outlining a research protocol for developing insect disinfestation treatments. By standardizing methods, this protocol helps researchers compare results and determine generic irradiation doses for different pest taxa. International standards are needed for establishing the efficacy of other treatment types and for other pest groups. New Zealand has published standards for the determination of fruit fly disinfestation treatment efficacy (102) and for the determination of fruit fly host status as a treatment (103). Similar international standards for these and other mitigation approaches would be beneficial. For example, standardized research protocols would help in establishing and maintaining low prevalence areas and in studying the infestation biology of pests to provide information for commodity pest risk assessments.

CONCLUSIONS

With world trade in agricultural commodities increasing, the introduction of exotic insects into new areas where they become pests will increase. Quarantine treatments or other mitigation approaches outlined in this review reduce or eliminate pest load in traded commodities and represent the best method to safeguard agriculture and natural resources worldwide. A single postharvest treatment applied to the commodity will remain the mainstay for trade in many commodities, but a range of alternative analytical techniques and mitigation options are available to prevent exotic pest introductions. Chemically based postharvest treatments will likely become less available and will be replaced by physical treatments and systems approaches. Developing systems approaches with sets of safeguards and mitigation measures requires better knowledge of pest biology, host plant interactions, and pest management than do traditional postharvest approaches. Regulators should solicit input from research biologists when conducting risk assessments and preparing regulations concerning quarantine pests. Designing postharvest treatments and systems approaches for taxonomic groups or guilds of insects and groups of commodities rather than for individual pests and commodities would help avoid research, regulatory, and trade bottlenecks. In support of PFA, systems approach, pest suppression, and pest eradication technologies, further research into developing optimal trapping designs for low-level populations, improving pheromone or plant-based lures, understanding insect dispersal, and integrating area-wide pest suppression tactics is needed in addition to studies of the ecological limitations of pests and improved pest risk assessment methods. The development of additional national and international standards for phytosanitary measures is needed to improve uniformity and transparency of information exchanged between countries when negotiating trade in new commodities.

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